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Antimicrobial Susceptibility Patterns of Anaerobic Bacterial Clinical Isolates From 2014 to 2016, Including Recently Named or Renamed Species

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Background: Anaerobic bacterial resistance trends may vary across regions or institutions. Regional susceptibility patterns are pivotal in the empirical treatment of anaerobic infections. We determined the antimicrobial resistance patterns of clinically important anaerobic bacteria, including recently named or renamed anaerobes.

Methods: A total of 521 non-duplicated clinical isolates of anaerobic bacteria were collected from a tertiary-care hospital in Korea between 2014 and 2016. Anaerobes were isolated from blood, body fluids, and abscess specimens. Each isolate was identified by conventional methods and by Bruker biotyper mass spectrometry (Bruker Daltonics, Leipzig, Germany) or VITEK matrix-assisted laser desorption ionization time-of-flight mass spectrometry (bioMérieux, Marcy-l'Étoile, France). Antimicrobial susceptibility was tested using the agar dilution method according to the CLSI guidelines. The following antimicrobials were tested: piperacillin-tazobactam, cefoxitin, cefotetan, imipenem, meropenem, clindamycin, moxifloxacin, chloramphenicol, tetracycline, and metronidazole.

Results: Most *Bacteroides fragilis* isolates were susceptible to piperacillin-tazobactam, imipenem, and meropenem. The non-*fragilis* *Bacteroides* group (including *B. intestinalis*, *B. nordii*, *B. pyogenes*, *B. stercoris*, *B. salyersiae*, and *B. cellulosilyticus*) was resistant to meropenem (14%) and cefotetan (71%), and *Parabacteroides distasonis* was resistant to imipenem (11%) and cefotetan (95%). Overall, the *Prevotella* and *Fusobacterium* isolates were more susceptible to antimicrobial agents than the *B. fragilis* group isolates. Anaerobic gram-positive cocci exhibited various resistance rates to tetracycline (6–86%). *Clostridioides difficile* was highly resistant to penicillin, cefoxitin, imipenem, clindamycin, and moxifloxacin.

Conclusions: Piperacillin-tazobactam, cefoxitin, and carbapenems are highly active β -lactam agents against most anaerobes, including recently named or renamed species.


Key Words: Antimicrobial resistance pattern, Anaerobes, *Bacteroides*, Korea

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INTRODUCTION

The prevalence of antibiotic resistance in anaerobes is increasing, which impacts both antibiotic treatment and patient mortality [1]. Regional susceptibility patterns are pivotal in the empiri-

cal treatment of anaerobic infections. As the resistance trends of anaerobic bacteria may vary greatly, across regions or institutions [2-4], antimicrobial susceptibility tests (ASTs) should be performed to assist with empirical antimicrobial treatment of anaerobic infections.

The CLSI has stated that routine ASTs for anaerobes are not necessary, because antibiotic resistance is often predictable [5]. Therefore, we do not always perform ASTs; however, since 1989, we have been performing periodic ASTs to investigate resistance trends among clinical bacterial isolates [6-9].

Anaerobic gram-negative bacilli (GNB) are clinically important because they have high resistance rates relative to other anaerobic bacteria [10]. Recently, a related cluster of multidrug-resistant *Bacteroides fragilis* isolates were recovered from several patients, which resulted in treatment failure in some cases [11, 12]. Furthermore, a number of anaerobic species have recently been named or renamed. *Parabacteroides distasonis* and *P. goldsteinii* were reclassified from the genus *Bacteroides*; *Alloscardovia omnicolens*, *Bulleidia extructa*, *Leptotrichia trevisanii*, *Alistipes finegoldii*, and *Alistipes onderdonkii* were named in the 2000s [13-18]. Moreover, AST data for infrequently isolated species are quite limited. Therefore, we collected rarely isolated anaerobic bacteria from clinical specimens and evaluated them using ASTs. In addition, we determined the antimicrobial resistance patterns of clinically important anaerobic bacteria, including recently named or renamed anaerobes.

METHODS

Bacterial isolates

A total of 521 non-duplicated clinical anaerobic bacteria isolates were collected from a tertiary-care hospital (Severance Hospital, Seoul, Korea) between 2014 and 2016. Anaerobes were isolated from blood, body fluids, and abscess specimens. Each isolate was identified by conventional methods, Bruker biotyper mass spectrometry (Bruker Daltonics, Leipzig, Germany), or VITEK matrix-assisted laser desorption ionization time-of-flight mass spectrometry (bioMérieux, Marcy-l'Étoile, France).

We tested a total of 230 gram-negative isolates, including 60 *Bacteroides fragilis*, 68 non-*fragilis* *Bacteroides* spp., 29 *Parabacteroides* spp., 33 *Prevotella* spp., 19 *Fusobacterium* spp., 10 other anaerobic GNB, and 11 *Veillonella* spp. Non-*fragilis* *Bacteroides* isolates were divided into two groups as follows: Group I included *B. thetaiotaomicron*, *B. caccae*, *B. uniformis*, *B. vulgatus*, and *B. ovatus*; Group II were recently classified, renamed, or infrequently isolated including *B. intestinalis*, *B. nordii*, *B. pyogenes*, *B. stercoris*, *B. salyersiae*, and *B. cellulosilyticus*. A total of 291 gram-positive isolates were tested, including 31 *Finegoldia magna*, 29 *Parvimonas micra*, 14 other gram-positive cocci (GPC), 15 *Clostridioides difficile*, 27 *Clostridium* spp., 34 *Actinomyces odontolyticus*, 23 *Actinomyces* spp., 18

Bifidobacterium spp., 38 *Eggerthella lenta*, 36 *Lactobacillus* spp., and 26 other gram-positive bacilli.

ASTs

ASTs were conducted using the agar dilution method, and minimum inhibitory concentrations (MICs) were interpreted according to the CLSI guidelines [5, 19]. The medium used was Brucella agar (Becton Dickinson, Cockeysville, MD, USA) supplemented with 5 µg/mL hemin, 1 µg/mL vitamin K₁, and 5% laked sheep blood. The following antimicrobials were tested: penicillin (Sigma Aldrich, Yongin, Korea), piperacillin-tazobactam (Yuhan, Seoul, Korea), cefoxitin (Merck Sharp & Dohme, West Point, PA, USA), cefotetan (Daiichi Pharmaceutical, Tokyo, Japan), imipenem and meropenem (Choongwae, Seoul, Korea), clindamycin (Korea Upjohn, Seoul, Korea), meropenem (Sumitomo, Tokyo, Japan), moxifloxacin (Bayer Korea, Seoul, Korea), chloramphenicol (Chong Kun Dang, Seoul, Korea), and tetracycline (Sigma Aldrich). For the piperacillin and tazobactam combination, a constant concentration of tazobactam (4 µg/mL) was added. An inoculum of 10⁵ colony forming units (CFUs) was applied with a Steers replicator (Craft Machine Inc., Woodline, PA, USA), and the plates were incubated in an anaerobic chamber (Forma Scientific, Marietta, OH, USA) for 48 hours at 37°C. Quality control was tested with the following two organisms: *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741. Double-disk potentiation tests (DPTs) with dipicolinic acid were carried out on Brucella agar to screen for carbapenemase-producing *B. fragilis* group isolates [20].

RESULTS

Anaerobic gram-negative isolates

Most of the gram-negative isolates tested were susceptible to piperacillin-tazobactam, imipenem, and meropenem, as their resistance rates to these three antimicrobials were <7% (Table 1). Low frequencies of resistance to chloramphenicol and metronidazole were observed for most of the anaerobic gram-negative bacterial isolates tested.

High rates of resistance to penicillin (98–100%), cefotetan (12–71%), and clindamycin (38–69%) were noted for the *B. fragilis* group isolates. The resistance of *B. fragilis* isolates to cefotetan was 12%; however, the non-*fragilis* *Bacteroides* Group II isolates showed high resistance to cefotetan (71%). Furthermore, *Parabacteroides* spp. (including *P. distasonis*), reclassified from the genus *Bacteroides*, showed very high resistance to cefotetan (95–100%). The resistance of *B. fragilis* and non-*fra-*

Table 1. Antimicrobial susceptibility of 521 anaerobic bacterial isolates from 2014 to 2016

| N of isolates and antimicrobial agents | Breakpoint (μg/mL) | | | MIC (μg/mL) | | | Susceptibility (%)* | | |
|--|--------------------|----|------|-------------|------|------|---------------------|----|-----|
| | S | I | R | Range | 50% | 90% | S | I | R |
| <i>Bacteroides fragilis</i> (60) | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | 4–>128 | 16 | >128 | 0 | 0 | 100 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | 0.12–>128 | 1 | 4 | 95 | 0 | 5 |
| Cefoxitin | ≤16 | 32 | ≥64 | 4–64 | 8 | 32 | 82 | 12 | 7 |
| Cefotetan | ≤16 | 32 | ≥64 | 2–>128 | 8 | 64 | 75 | 13 | 12 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–32 | 0.12 | 1 | 95 | 0 | 5 |
| Meropenem | ≤4 | 8 | ≥16 | ≤0.06–>128 | 0.12 | 2 | 92 | 3 | 5 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–>128 | 1 | >128 | 60 | 2 | 38 |
| Moxifloxacin | ≤2 | 4 | ≥8 | ≤0.06–32 | 0.5 | 8 | 77 | 3 | 20 |
| Chloramphenicol | ≤8 | 16 | ≥32 | 4–8 | 4 | 8 | 100 | 0 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.25–8 | 4 | 4 | 100 | 0 | 0 |
| Non- <i>fragilis</i> <i>Bacteroides</i> group I (54) [†] | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–>128 | 128 | >128 | 2 | 0 | 98 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–>128 | 8 | 32 | 93 | 2 | 6 |
| Cefoxitin | ≤16 | 32 | ≥64 | 1–>128 | 16 | 32 | 57 | 35 | 7 |
| Cefotetan | ≤16 | 32 | ≥64 | 0.5–>128 | 64 | >128 | 17 | 24 | 59 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–32 | 0.5 | 2 | 94 | 4 | 2 |
| Meropenem | ≤4 | 8 | ≥16 | ≤0.06–4 | 0.5 | 2 | 100 | 0 | 0 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–>128 | >128 | >128 | 20 | 11 | 69 |
| Moxifloxacin | ≤2 | 4 | ≥8 | ≤0.06–32 | 2 | 8 | 78 | 7 | 15 |
| Chloramphenicol | ≤8 | 16 | ≥32 | 2–8 | 8 | 8 | 100 | 0 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.5–8 | 2 | 4 | 100 | 0 | 0 |
| Non- <i>fragilis</i> <i>Bacteroides</i> group II (14) [‡] | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | 16–>128 | 16 | >128 | 0 | 0 | 100 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | 0.5–32 | 8 | 32 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | 1–64 | 32 | 32 | 43 | 50 | 7 |
| Cefotetan | ≤16 | 32 | ≥64 | 4–>128 | 64 | 128 | 21 | 7 | 71 |
| Imipenem | ≤4 | 8 | ≥16 | 0.12–2 | 0.25 | 2 | 100 | 0 | 0 |
| Meropenem | ≤4 | 8 | ≥16 | 0.12–32 | 0.25 | 16 | 86 | 0 | 14 |
| Clindamycin | ≤2 | 4 | ≥8 | 0.5–>128 | >128 | >128 | 36 | 0 | 64 |
| Moxifloxacin | ≤2 | 4 | ≥8 | 0.5–64 | 1 | 16 | 79 | 0 | 21 |
| Chloramphenicol | ≤8 | 16 | ≥32 | 4–8 | 8 | 8 | 100 | 0 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | 2–4 | 2 | 4 | 100 | 0 | 0 |
| <i>Parabacteroides distasonis</i> (19) | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–>128 | >128 | >128 | 5 | 0 | 95 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–>128 | 32 | >128 | 89 | 0 | 11 |
| Cefoxitin | ≤16 | 32 | ≥64 | 1–128 | 32 | 64 | 21 | 42 | 37 |
| Cefotetan | ≤16 | 32 | ≥64 | 1–>128 | 128 | >128 | 5 | 0 | 95 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–64 | 1 | 16 | 89 | 0 | 11 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–>128 | >128 | >128 | 5 | 16 | 79 |

(Continued to the next page)

Table 1. Continued

| N of isolates and antimicrobial agents | Breakpoint (μg/mL) | | | MIC (μg/mL) | | | Susceptibility (%) [*] | | |
|--|--------------------|----|------|-------------|-------|-------|---------------------------------|----|-----|
| | S | I | R | Range | 50% | 90% | S | I | R |
| Moxifloxacin | ≤2 | 4 | ≥8 | 0.12–32 | 0.5 | 16 | 79 | 0 | 21 |
| Chloramphenicol | ≤8 | 16 | ≥32 | 2–8 | 8 | 8 | 100 | 0 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.5–4 | 2 | 4 | 100 | 0 | 0 |
| <i>Parabacteroides</i> spp. (10) [§] | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | 8–>128 | >128 | >128 | 0 | 0 | 100 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | 2–32 | 16 | 32 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | 16–64 | 32 | 64 | 20 | 50 | 30 |
| Cefotetan | ≤16 | 32 | ≥64 | 64–>128 | 128 | >128 | 0 | 0 | 100 |
| Imipenem | ≤4 | 8 | ≥16 | 1–4 | 1 | 4 | 100 | 0 | 0 |
| Clindamycin | ≤2 | 4 | ≥8 | 0.5–>128 | >128 | >128 | 20 | 0 | 80 |
| Moxifloxacin | ≤2 | 4 | ≥8 | 0.25–16 | 0.5 | 16 | 60 | 10 | 30 |
| Chloramphenicol | ≤8 | 16 | ≥32 | 4–8 | 8 | 8 | 100 | 0 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | 1–4 | 2 | 4 | 100 | 0 | 0 |
| <i>Prevotella</i> spp. (33) [‡] | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–>128 | 16 | 32 | 6 | 3 | 91 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–8 | ≤0.06 | ≤0.06 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | 0.5–32 | 1 | 4 | 97 | 3 | 0 |
| Cefotetan | ≤16 | 32 | ≥64 | 0.5–64 | 2 | 32 | 88 | 9 | 3 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–1 | ≤0.06 | ≤0.06 | 100 | 0 | 0 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–>128 | ≤0.06 | >128 | 55 | 0 | 45 |
| Moxifloxacin | ≤2 | 4 | ≥8 | 0.12–64 | 0.5 | 4 | 70 | 21 | 9 |
| Chloramphenicol | ≤8 | 16 | ≥32 | 1–16 | 2 | 8 | 91 | 9 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.12–32 | 1 | 8 | 91 | 6 | 3 |
| <i>Fusobacterium</i> spp.(19) [¶] | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–>128 | 0.25 | 4 | 79 | 5 | 16 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–8 | 2 | 4 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | 0.12–16 | 4 | 8 | 100 | 0 | 0 |
| Cefotetan | ≤16 | 32 | ≥64 | ≤0.06–32 | 2 | 4 | 95 | 5 | 0 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–4 | 1 | 2 | 100 | 0 | 0 |
| Meropenem | ≤4 | 8 | ≥16 | ≤0.06–2 | ≤0.06 | 1 | 100 | 0 | 0 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–128 | 2 | 16 | 58 | 21 | 21 |
| Moxifloxacin | ≤2 | 4 | ≥8 | ≤0.06–128 | 4 | 8 | 42 | 47 | 11 |
| Chloramphenicol | ≤8 | 16 | ≥32 | ≤0.06–2 | 2 | 2 | 100 | 0 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.12–1 | ≤0.06 | 1 | 100 | 0 | 0 |
| Other gram-negative bacilli (10) ^{**} | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–>128 | 1 | 16 | 30 | 30 | 40 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–>128 | 1 | 128 | 80 | 0 | 20 |
| Cefoxitin | ≤16 | 32 | ≥64 | 0.25–32 | 2 | 32 | 80 | 20 | 0 |
| Cefotetan | ≤16 | 32 | ≥64 | 0.5–32 | 2 | 4 | 90 | 10 | 0 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–0.5 | 0.25 | 0.25 | 100 | 0 | 0 |

(Continued to the next page)

Table 1. Continued

| N of isolates and antimicrobial agents | Breakpoint (μg/mL) | | | MIC (μg/mL) | | | Susceptibility (%) [*] | | |
|---|--------------------|----|------|-------------|-------|-------|---------------------------------|----|-----|
| | S | I | R | Range | 50% | 90% | S | I | R |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–32 | ≤0.06 | 4 | 90 | 0 | 10 |
| Moxifloxacin | ≤2 | 4 | ≥8 | ≤0.06–16 | 0.5 | 16 | 50 | 10 | 40 |
| Chloramphenicol | ≤8 | 16 | ≥32 | 0.25–8 | 4 | 8 | 100 | 0 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | ≤0.06–64 | NA | NA | NA | NA | NA |
| <i>Veillonella</i> spp. (11) ^{††} | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | 2–16 | 4 | 16 | 0 | 0 | 100 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | 4–128 | 16 | 32 | 91 | 0 | 9 |
| Cefoxitin | ≤16 | 32 | ≥64 | 2–8 | 4 | 8 | 100 | 0 | 0 |
| Cefotetan | ≤16 | 32 | ≥64 | 0.5–32 | 1 | 2 | 91 | 9 | 0 |
| Imipenem | ≤4 | 8 | ≥16 | 0.25–8 | 0.50 | 2 | 91 | 9 | 0 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–>128 | ≤0.06 | 2 | 91 | 0 | 9 |
| Moxifloxacin | ≤2 | 4 | ≥8 | ≤0.06–64 | 0.25 | 4 | 82 | 9 | 9 |
| Chloramphenicol | ≤8 | 16 | ≥32 | 0.5–2 | 2 | 2 | 100 | 0 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | 2–32 | 8 | 32 | 73 | 0 | 27 |
| <i>Finegoldia magna</i> (31) | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–0.12 | ≤0.06 | ≤0.06 | 100 | 0 | 0 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–0.12 | ≤0.06 | ≤0.06 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | 0.25–4 | 0.5 | 2 | 100 | 0 | 0 |
| Cefotetan | ≤16 | 32 | ≥64 | 0.12–4 | 0.25 | 2 | 100 | 0 | 0 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–≤0.06 | ≤0.06 | ≤0.06 | 100 | 0 | 0 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–64 | ≤0.06 | 0.5 | 94 | 3 | 3 |
| Moxifloxacin | ≤2 | 4 | ≥8 | 0.12–8 | 0.25 | 0.5 | 94 | 0 | 6 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.12–8 | 1 | 1 | 100 | 0 | 0 |
| Tetracycline | ≤4 | 8 | ≥16 | ≤0.06–16 | 0.25 | 4 | 94 | 0 | 6 |
| <i>Parvimonas micra</i> (29) | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–0.25 | 0.12 | 0.25 | 100 | 0 | 0 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–2 | 0.12 | 0.25 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | 0.25–4 | 0.5 | 1 | 100 | 0 | 0 |
| Cefotetan | ≤16 | 32 | ≥64 | 0.5–2 | 1 | 2 | 100 | 0 | 0 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–0.25 | ≤0.06 | 0.12 | 100 | 0 | 0 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–128 | 1 | 128 | 76 | 0 | 24 |
| Moxifloxacin | ≤2 | 4 | ≥8 | ≤0.06–32 | 2 | 32 | 52 | 0 | 48 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.5–4 | 1 | 2 | 100 | 0 | 0 |
| Tetracycline | ≤4 | 8 | ≥16 | 1–64 | 16 | 32 | 45 | 0 | 55 |
| Other gram-positive cocci (14) [†] | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–8 | 0.12 | 8 | 64 | 0 | 36 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–16 | 0.25 | 16 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | ≤0.06–16 | 0.50 | 16 | 100 | 0 | 0 |
| Cefotetan | ≤16 | 32 | ≥64 | 0.25–128 | 4 | 128 | 50 | 7 | 43 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–4 | 0.25 | 4 | 100 | 0 | 0 |

(Continued to the next page)

Table 1. Continued

| N of isolates and antimicrobial agents | Breakpoint (µg/mL) | | | MIC (µg/mL) | | | Susceptibility (%) [*] | | |
|--|--------------------|----|------|-------------|-------|------|---------------------------------|----|-----|
| | S | I | R | Range | 50% | 90% | S | I | R |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–128 | 0.25 | 128 | 50 | 7 | 43 |
| Moxifloxacin | ≤2 | 4 | ≥8 | 0.12–16 | 2 | 8 | 64 | 7 | 29 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.5–8 | 2 | 2 | 100 | 0 | 0 |
| Tetracycline | ≤4 | 8 | ≥16 | 0.25–64 | 32 | 64 | 14 | 0 | 86 |
| <i>Clostridioides difficile</i> (15) | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | 2–4 | 2 | 4 | 0 | 0 | 100 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | 4–16 | 16 | 16 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | 128–>128 | 128 | >128 | 0 | 0 | 100 |
| Cefotetan | ≤16 | 32 | ≥64 | 16–64 | 32 | 64 | 20 | 40 | 40 |
| Imipenem | ≤4 | 8 | ≥16 | 4–64 | 16 | 32 | 7 | 0 | 93 |
| Clindamycin | ≤2 | 4 | ≥8 | 1–>128 | 16 | >128 | 7 | 27 | 67 |
| Moxifloxacin | ≤2 | 4 | ≥8 | 1–32 | 16 | 32 | 47 | 0 | 53 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.5–4 | 2 | 2 | 100 | 0 | 0 |
| Tetracycline | ≤4 | 8 | ≥16 | 0.25–32 | 0.5 | 32 | 60 | 13 | 27 |
| <i>Clostridium</i> spp. (27) ⁱ | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–2 | 0.5 | 2 | 74 | 15 | 11 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–32 | 0.5 | 16 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | 0.25–128 | 2 | 64 | 85 | 4 | 11 |
| Cefotetan | ≤16 | 32 | ≥64 | 0.25–>128 | 4 | >128 | 78 | 4 | 19 |
| Imipenem | ≤4 | 8 | ≥16 | 0.25–8 | 1 | 4 | 96 | 4 | 0 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–>128 | 1 | >128 | 63 | 4 | 33 |
| Moxifloxacin | ≤2 | 4 | ≥8 | 0.12–128 | 1 | 32 | 74 | 7 | 19 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.25–64 | 2 | 8 | 93 | 0 | 7 |
| Tetracycline | ≤4 | 8 | ≥16 | 0.12–64 | 16 | 64 | 26 | 11 | 63 |
| <i>Actinomyces odontolyticus</i> (34) | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–8 | 0.5 | 8 | 53 | 18 | 29 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | 0.5–64 | 4 | 32 | 91 | 9 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | ≤0.06–32 | 1 | 16 | 97 | 3 | 0 |
| Cefotetan | ≤16 | 32 | ≥64 | 0.5–128 | 8 | 128 | 65 | 12 | 24 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–8 | 0.5 | 2 | 97 | 3 | 0 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–>128 | 0.5 | >128 | 62 | 0 | 38 |
| Moxifloxacin | ≤2 | 4 | ≥8 | 2–32 | 2 | 2 | 97 | 0 | 3 |
| Metronidazole | ≤8 | 16 | ≥32 | 8–>128 | 32 | >128 | 6 | 29 | 65 |
| Tetracycline | ≤4 | 8 | ≥16 | 2–32 | 2 | 16 | 79 | 0 | 21 |
| <i>Actinomyces</i> spp. (23) ⁱⁱ | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–0.5 | 0.12 | 0.12 | 100 | 0 | 0 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–1 | 0.5 | 1 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | 0.12–1 | 0.25 | 1 | 100 | 0 | 0 |
| Cefotetan | ≤16 | 32 | ≥64 | ≤0.06–4 | 0.5 | 4 | 100 | 0 | 0 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–0.25 | ≤0.06 | 0.25 | 100 | 0 | 0 |

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Table 1. Continued

| N of isolates and antimicrobial agents | Breakpoint (μg/mL) | | | MIC (μg/mL) | | | Susceptibility (%) [*] | | |
|--|--------------------|----|------|-------------|-------|------|---------------------------------|----|-----|
| | S | I | R | Range | 50% | 90% | S | I | R |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–>128 | 0.25 | >128 | 78 | 0 | 22 |
| Moxifloxacin | ≤2 | 4 | ≥8 | 0.5–2 | 1 | 2 | 100 | 0 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | 32–>128 | >128 | >128 | 0 | 0 | 100 |
| Tetracycline | ≤4 | 8 | ≥16 | 0.5–64 | 1 | 32 | 78 | 0 | 22 |
| <i>Bifidobacterium</i> spp. (18) ^{¶¶} | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–4 | 0.12 | 4 | 72 | 11 | 17 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–32 | 0.12 | 16 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | ≤0.06–64 | 1 | 64 | 83 | 0 | 17 |
| Cefotetan | ≤16 | 32 | ≥64 | 0.25–>128 | 2 | >128 | 72 | 0 | 28 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–1 | 0.12 | 0.5 | 100 | 0 | 0 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–>128 | 0.5 | >128 | 72 | 0 | 28 |
| Moxifloxacin | ≤2 | 4 | ≥8 | ≤0.06–16 | 1 | 4 | 89 | 6 | 6 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.5–>128 | 8 | >128 | 67 | 11 | 22 |
| Tetracycline | ≤4 | 8 | ≥16 | 2–128 | 2 | 16 | 83 | 6 | 11 |
| <i>Eggerthella lenta</i> (38) | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | 0.5–2 | 1 | 2 | 8 | 45 | 47 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | 16–32 | 16 | 32 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | 2–32 | 8 | 16 | 95 | 5 | 0 |
| Cefotetan | ≤16 | 32 | ≥64 | 32–>128 | 128 | >128 | 0 | 5 | 95 |
| Imipenem | ≤4 | 8 | ≥16 | 0.5–0.5 | 0.5 | 1 | 100 | 0 | 0 |
| Clindamycin | ≤2 | 4 | ≥8 | 0.12–0.5 | 0.5 | >128 | 63 | 0 | 37 |
| Moxifloxacin | ≤2 | 4 | ≥8 | 0.12–4 | 4 | 64 | 47 | 21 | 32 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.5–1 | 1 | 1 | 100 | 0 | 0 |
| Tetracycline | ≤4 | 8 | ≥16 | 0.5–32 | 32 | 64 | 37 | 3 | 61 |
| <i>Lactobacillus</i> spp. (36) ^{***} | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–>128 | 0.5 | 2 | 56 | 22 | 22 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | 0.5–>128 | 4 | 8 | 94 | 0 | 6 |
| Cefoxitin | ≤16 | 32 | ≥64 | 4–>128 | >128 | >128 | 17 | 3 | 81 |
| Cefotetan | ≤16 | 32 | ≥64 | 8–>128 | >128 | >128 | 3 | 0 | 97 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–16 | 0.25 | 8 | 86 | 11 | 3 |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–1 | 0.12 | 0.5 | 100 | 0 | 0 |
| Moxifloxacin | ≤2 | 4 | ≥8 | 0.25–4 | 1 | 2 | 94 | 6 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | 32–>128 | >128 | >128 | 0 | 0 | 100 |
| Tetracycline | ≤4 | 8 | ≥16 | 0.5–>128 | 8 | 32 | 44 | 33 | 22 |
| Other gram-positive bacilli (26) ^{††} | | | | | | | | | |
| Penicillin | ≤0.5 | 1 | ≥2 | ≤0.06–4 | 0.12 | 0.25 | 96 | 0 | 4 |
| Piperacillin-tazobactam | ≤32 | 64 | ≥128 | ≤0.06–2 | 0.12 | 2 | 100 | 0 | 0 |
| Cefoxitin | ≤16 | 32 | ≥64 | ≤0.06–16 | 1 | 4 | 100 | 0 | 0 |
| Cefotetan | ≤16 | 32 | ≥64 | ≤0.06–32 | 2 | 8 | 96 | 4 | 0 |
| Imipenem | ≤4 | 8 | ≥16 | ≤0.06–0.5 | ≤0.06 | 0.12 | 100 | 0 | 0 |

(Continued to the next page)

Table 1. Continued

| N of isolates and antimicrobial agents | Breakpoint (μg/mL) | | | MIC (μg/mL) | | | Susceptibility (%) [*] | | |
|--|--------------------|----|-----|-------------|-------|------|---------------------------------|----|----|
| | S | I | R | Range | 50% | 90% | S | I | R |
| Clindamycin | ≤2 | 4 | ≥8 | ≤0.06–64 | ≤0.06 | 4 | 85 | 8 | 8 |
| Moxifloxacin | ≤2 | 4 | ≥8 | ≤0.06–4 | 0.25 | 1 | 96 | 4 | 0 |
| Metronidazole | ≤8 | 16 | ≥32 | 0.25–>128 | 8 | >128 | 60 | 0 | 40 |
| Tetracycline | ≤4 | 8 | ≥16 | 0.25–8 | 2 | 8 | 72 | 28 | 0 |

^{*}Susceptibility was determined by breakpoint according to the CLSI M100 27th edition [19]; [†]*Bacteroides thetaiotaomicron* (N=26), *B. caccae* (N=9), *B. uniformis* (N=7), *B. vulgatus* (N=7), *B. ovatus* (N=5); [‡]*B. intestinalis* (N=4), *B. nordii* (N=3), *B. pyogenes* (N=2), *B. stercoris* (N=2), *B. salyersiae* (N=2), *B. cellulosilyticus* (N=1); [§]*Parabacteroides goldsteinii* (N=5), *P. johnsonii* (N=2), *P. merdae* (N=2), *P. faecis* (N=1); ^{||}*Prevotella buccae* (N=15), *P. bivia* (N=10), *P. nigrescens* (N=3), *P. buccalis* (N=1), *P. disiens* (N=1), *P. intermedia* (N=1), *P. melaninogenica* (N=1), *P. oralis* (N=1); [¶]*Fusobacterium vari-um* (N=14), *F. mortiferum* (N=2), *F. ulcerans* (N=2), *F. nucleatum* (N=1); ^{**}*Dialister pneumosintes* (N=2), *Leptotrichia trevisanii* (N=2), *L. buccalis* (N=1), *Alistipes finegoldii* (N=1), *A. onderdonkii* (N=1), *Bilophila* sp. (N=1), *Megamonas* sp. (N=1), *Sutterella wadsworthensis* (N=1); ^{††}*Veillonella parvu-la* (N=9), *V. atypica* (N=1), *V. dispar* (N=1); ^{‡‡}*Peptoniphilus anaerobius* (N=3), *P. asaccharolyticus* (N=2), *P. gorbachii* (N=2), *P. harei* (N=1), *Anaero-coccus vaginalis* (N=2), *A. murdochii* (N=1), *A. prevotii* (N=1), *Ruminococcus gnavus* (N=2); ^{§§}*Clostridium bifermentans* (N=3), *C. hathewayi* (N=3), *C. innocuum* (N=3), *C. paraputrificum* (N=3), *C. perfringens* (N=3), *C. butyricum* (N=2), *C. ramosum* (N=2), *C. sordellii* (N=2), *C. tertium* (N=2), *C. ca-daveris* (N=1), *C. scindens* (N=1), *C. sporogenes* (N=1), *C. bolteae* (N=1); ^{|||}*Actinomyces oris* (N=7), *A. turicensis* (N=7), *A. neuui* (N=4), *A. viscosus* (N=2), *A. europaeus* (N=1), *A. meyeri* (N=1), *A. naeslundii* (N=1); ^{¶¶}*Bifidobacterium dentium* (N=5), *B. longum* (N=5), *B. breve* (N=4), *B. bifidum* (N=2), *B. pseudocatenulatum* (N=1), *B. thermophilum* (N=1); ^{***}*Lactobacillus paracasei* (N=5), *L. rhamnosus* (N=5), *L. sakei* (N=5), *L. salivarius* (N=4), *L. fermentum* (N=3), *L. mucosae* (N=3), *L. crispatus* (N=2), *L. gasseri* (N=2), *L. plantarum* (N=2), *L. reuteri* (N=2), *L. curvatus* (N=1), *L. harbinensis* (N=1), *L. sporogenes* (N=1); ^{†††}*Atopobium parvulum* (N=7), *A. rimae* (N=2), *Propionibacterium acnes* (N=5), *P. avidum* (N=1), *P. lymphophilum* (N=1), *Ac-tinotignum schaalii* (N=2), *Alloscardovia omnicolens* (N=2), *Bulleidia extructa* (N=2), *Collinsella aerofaciens* (N=2), *Flavonifractor plautii* (N=1), *Slackia exigua* (N=1).

Abbreviations: S, susceptible; I, intermediate; R, resistant; MIC, minimum inhibitory concentration.

gilis Bacteroides group I and II isolates to moxifloxacin was 20% and 16%, respectively. Overall, *Parabacteroides* spp. exhibited higher resistance rates relative to *B. fragilis* spp., especially for clindamycin (79%) and moxifloxacin (24%). *Bacteroides fragilis* exhibited imipenem and meropenem-resistance rates of 5%. Non-*fragilis Bacteroides* Group I showed resistance to only imipenem (2%), while non-*fragilis Bacteroides* Group II showed resistance to only meropenem (14%). The meropenem MIC required to decrease growth by 90% (MIC₉₀=16 μg/mL) for non-*fragilis Bacteroides* Group II was higher than that for *B. fragilis* and non-*fragilis Bacteroides* Group I (MIC₉₀=2 μg/mL). Four carbapenem-non-susceptible *B. fragilis* isolates showed positive results on DPTs, whereas eight carbapenem-non-susceptible non-*fragilis Bacteroides* isolates (including *B. thetaiotaomicron*, *B. intestinalis*, *B. nordii*, *P. distasonis*, and *P. merdae*) showed negative results.

Overall, *Prevotella* and *Fusobacterium* isolates were more susceptible to antimicrobial agents than *B. fragilis* group isolates. Interestingly, one *Prevotella* spp. isolate was resistant to metronidazole (3%). The other anaerobic GNB were susceptible to most of the antibiotics tested. However, all *Leptotrichia* isolates were resistant to moxifloxacin (MIC=8–16 μg/mL). *Megamonas* spp. and *Sutterella wadsworthensis* were resistant to piperacillin-tazobactam (MIC ≥128 μg/mL), and three *Veillon-ella* isolates (27%) were resistant to metronidazole.

Anaerobic gram-positive isolates

A total of 74 anaerobic GPC, including 31 *Finegoldia magna* and 29 *Parvimonas micra*, exhibited various resistance rates to moxifloxacin (6–48%), clindamycin (3–43%), and tetracycline (6–86%). Overall, *F. magna* isolates were more susceptible than other GPC isolates, with a resistance rate <6% to all antimicro-bials tested (Table 1). The resistance rate of the other GPC iso-lates to penicillin was 36%, with all species identified as *Pepto-niphilus*.

C. difficile showed high resistance to penicillin (100%), cefox-itin (100%), imipenem (93%), and moxifloxacin (53%). All non-*odontolyticus Actinomyces* and *Lactobacillus* isolates and 65% of *Actinomyces odontolyticus* isolates were resistant to metroni-dazole. All non-*odontolyticus Actinomyces* isolates were suscep-tible to the other antimicrobial agents tested, except for clindamy-cin (22% resistance) and tetracycline (22% resistance). *E. lenta* demonstrated high resistance rates to penicillin (47%), cefotetan (95%), tetracycline (61%), and moxifloxacin (32%). Other GPB, such as *Actinotignum*, *Alloscardovia*, *Bulleidia*, *Collinsella*, *Fla-vonifractor*, and *Slackia*, were generally susceptible to all agents tested, except for metronidazole.

DISCUSSION

The *Bacteroides fragilis* group of anaerobic gram-negative iso-

lates (including *Parabacteroides* spp.) are the most clinically significant anaerobes because they are commonly isolated from clinical specimens and show greater virulence and resistance than most other anaerobes [10]. The resistance of *B. fragilis* isolates to cefotetan remained low for several years: 14% in 1997–2004 [8], 14% in 2007–2008 [7], 13% in 2009–2012 [9], and 12% in 2014–2016.

The resistance of *B. fragilis* isolates to moxifloxacin has steadily increased over the past 11 years, from 11% in 2007–2008 to 20% in 2014–2016. The current values are similar to those observed in 2010–2012 in the USA (19.1%) [21]. The resistance to moxifloxacin among non-*fragilis* *Bacteroides* group species has not increased; the rates have ranged from 18% in 2007–2008 to 16% in 2014–2016 [7]. This may reflect the fact that the *B. fragilis* group includes former members of the group previously reclassified as *Parabacteroides* spp. [7]. *Parabacteroides* spp. had a higher resistance rate to clindamycin and a lower resistance rate to moxifloxacin compared with isolates in the USA (50% and 44%, respectively) [21].

We observed that non-*fragilis* *Bacteroides* Group II had higher resistance rates to meropenem than imipenem, while non-*fragilis* *Bacteroides* Group I demonstrated the opposite pattern. Such patterns have been previously reported by S6ki *et al.* [22]; however, they did not include the carbapenem resistance patterns of non-*fragilis* *Bacteroides* Group II.

Prevotella spp. were highly susceptible to most antimicrobials except penicillin and clindamycin. The resistance rates to clindamycin remained high, at 45%, for *Prevotella* spp., compared with 50% in 2007–2008 [7]. Only one *Prevotella* spp. isolate was resistant to metronidazole. This represents an even lower rate of resistance than that reported in Greece (8%) [23]. The *Veillonella* resistance rate to metronidazole was 27%, higher than that reported in the USA (11%) [4].

The anaerobic GPC isolates exhibited various rates of resistance to penicillin, clindamycin, and metronidazole [2]. However, the resistance rate of GPC to clindamycin, moxifloxacin, and tetracycline varied across species. The resistance of *C. difficile* to imipenem has rapidly increased over the past years, from 8% in 2007–2008 to 93% in 2014–2016 [7]. There is a general assumption that resistance varies with ribotype; Lee *et al.* [24] showed that ribotypes O17 and O18 have high MICs for moxifloxacin and imipenem, compared with ribotype O01. Metronidazole-resistant isolates were common among *Actinomyces* and *Lactobacillus* spp. A study in Argentina showed that all *Actinomyces* spp. were susceptible to penicillin, and 21.2% were resistant to clindamycin [25]. *E. lenta* has been commonly as-

sociated with gastrointestinal infections; its overall mortality is significant, ranging from 36% to 48% [26, 27]. The *E. lenta* resistance rates we observed were much higher than those in Australia (0% for penicillin and 12% for moxifloxacin) [26].

The limitations of this study were the small number of re-named and reclassified bacteria and bacterial isolates collected. Further, it was a single-center, retrospective study.

In conclusion, piperacillin-tazobactam, cefoxitin, and carbapenems were β -lactam agents highly active against most of the anaerobic bacteria we tested. However, recently renamed non-*fragilis* *Bacteroides* group isolates showed resistance to meropenem (14%). These data suggest the importance of ongoing surveillance to provide clinically relevant information to clinicians for the empirical management of infections caused by anaerobic organisms. Continuous monitoring is necessary to detect changes in antimicrobial resistance patterns.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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